

# **Dynamics of p53 in response to DNA damage vary across cell lines and are shaped by efficiency of DNA repair and activity of the kinase ATM**

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## **Supplemental Text**

### **Model of p53 dynamics**

Simulation of p53 dynamics – implemented in Matlab (2014B)

#### Variables

*p53* – Concentration of p53 protein

*p53p* – Concentration of active (or phospho) p53

*mdm2* – Concentration of MDM2

*mdm2p* – Concentration of phospho-MDM2

Simulations were run with the following parameters – when n and nm were the parameters that were varied in figure 7.

```
k(1)=10; %transition to inactive p53  
k(2)=n; %transition to active p53  
k(3)=log(2)/0.05; %p53 deg rate mdm2  
k(4)=log(2)/5; %p53 deg rate  
k(5)=log(2)/5; %p53p deg rate mdm2  
k(6)=log(2)/5; %p53p deg rate  
k(7)=0.1; %MDM2 basal transcription  
k(8)=10; %MDM2 transcription by p53  
k(9)=log(2)/5; %alphap - decay rate of mdm2 (not mdm2 dependant)  
k(10)=log(2)/5; %decay rate of MDM2 (mdm2 cat)  
k(11)=1; %basal p53 transcription  
k(12)=40; %delay before ATM activity  
k(13)=0.25; %MDM2 affinity constant p53
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$k(14)=2.5$ ; %MDM2 affinity constant for pP53  
 $k(15)=0.25$ ; %MDM2 affinity constant for mdm2  
 $k(16)=\log(2)/0.02$ ; %mdm2 deg rate  
 $k(17)=\log(2)/0.02$ ; %mdm2p deg rate mdm2  
 $k(18)=nn$ ; %decay rate of ATM activity

$$dp53 = k11 + p53p * k1 - p53 * k2 - k2 * p53 * \left(\frac{mdm2s}{mdm2s+k13}\right)^3 - p53 * k4$$

$$dp53p = -p53p * k1 + p53 * k2 - k5 * p53 * \left(\frac{mdm2s}{mdm2s+k14}\right)^3 - p53 * k6$$

$$dmdm2 = k7 + mdm2p * k1 - mdm2p * k2 + k8 * (p53lag)^4 - k10 * mdm2 * \left(\frac{mdm2s}{mdm2s+k15}\right) - mdm2 * k9$$

$$dmdm2p = -mdm2p * k1 + mdm2p * k2 - k17 * mdm2 * \left(\frac{mdm2s}{mdm2s+k15}\right) - mdm2 * k17$$

ATM activity was modeled as

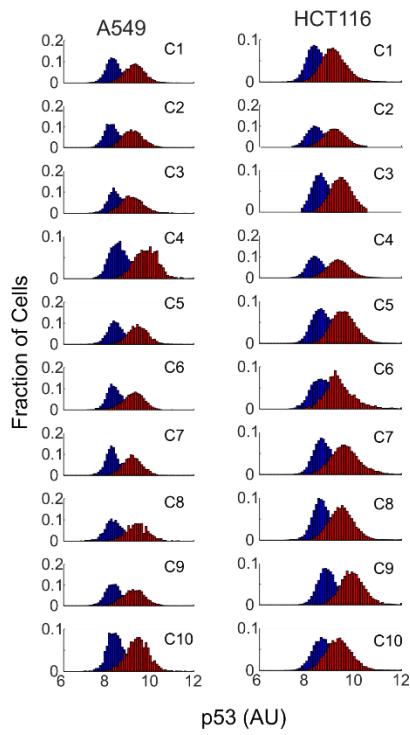
$$k2 = k2 * (\text{heaviside}(t - k12)) * \exp(-k18 * (t - k12))$$

$p53lag$  is the 2hr delayed concentration of p53+p53p

$mdm2s$  is the sum of mdm2 and mdm2p

## Supplemental Figures

**A**



**B**

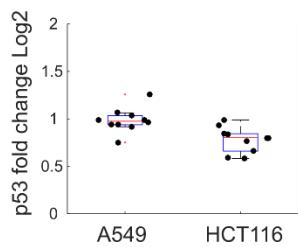


Figure S1: Cell-to-cell variation in p53 abundance is not due to genetic inhomogeneity. (A) p53 was stained and imaged in 10 clones of HCT116 and A549 before and after treatment with NCS (200ng/ml, 2hrs). Quantification of p53 is shown as histograms (N>1000 cells, pooled from experiments performed in triplicates). (B) Mean log<sub>2</sub> fold change of p53 intensity of treated versus untreated cells for each clone (single dots), with a box plot showing the overall behavior of the population of clones (N=10 clones).

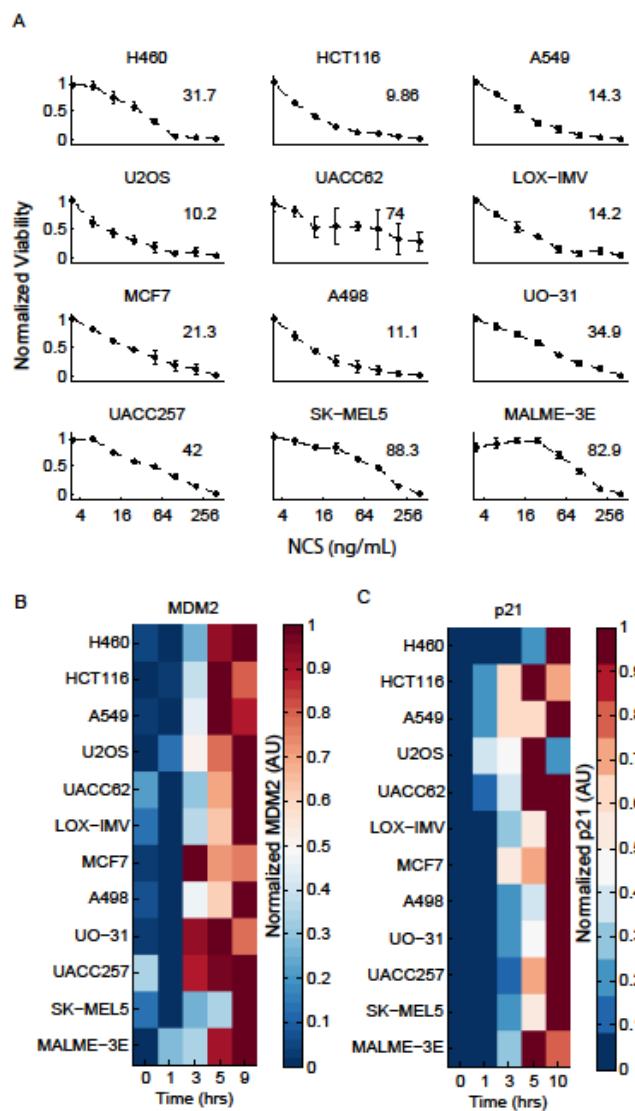


Figure S2: Proliferation and induction of p53 target genes after DNA damage across cell lines. (A) The twelve indicated cell lines were assayed for proliferation after treatment with a 8 point titration of NCS. The EC50 of each curve is noted in the upper right. (B, C) Measurement of mean MDM2

(B) or p21 (C) levels by IF in each cell line at the indicated time-points after NCS treatment (N>100 cells).

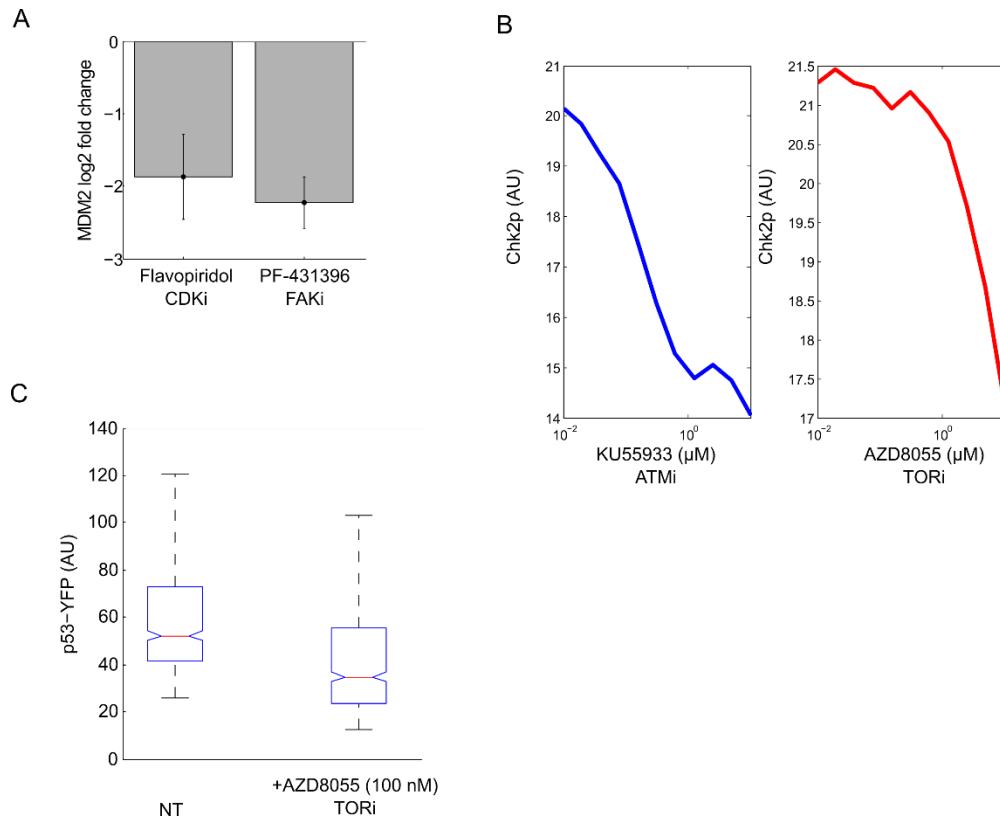


Figure S3: Characterization of modulators of p53 dynamics. (A) Treatment of MCF7 cells with Flavopiridol (CDKi, 2 $\mu$ M) or PF-431396 (FAKi, 2 $\mu$ M) for 3hrs reduces MDM2 RNA levels. Error bars represent S.E.M (N=3). (B) ATM activity in A549 cells measured by pCHK2 staining 30 minutes after treatment with 10Gy IR radiation and ATM (KU55933) or TOR (AZD8055) inhibitors (N>250 cells, mean of 3 exp). (C) p53-YFP in A549 cells in basal conditions and after 1hr of treatment with 100nM of the TOR inhibitor AZD8055 (N>250 cells, representative of 2 exp.).

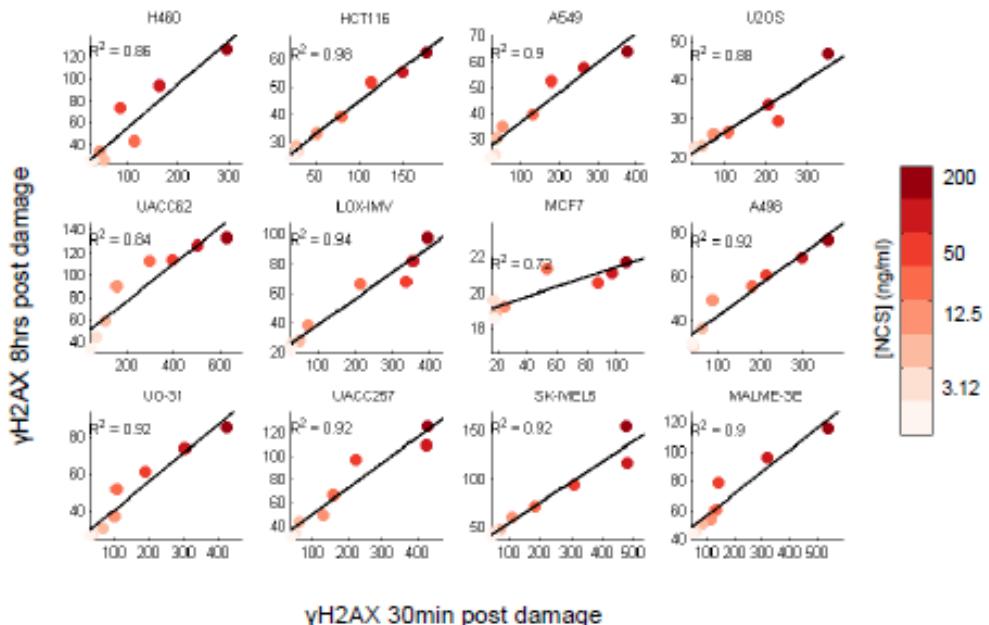


Figure S4: The fraction of unrepaired DNA breaks is cell-line specific and does not depend on the damage dose. (A) DNA damage quantified by phospho-H2AX ( $\gamma$ H2AX) was measured across the twelve cell lines at 30 min and 8hr across a 8-point titration of NCS (N>100 cells, representative of 2 exp.).

Table S1: Summary of chemical screening data. Compound, putative target, p53 IF intensity (AU), p53-YFP abundance at 4hrs (2μM dose, AU), p53-YFP intensity at 20hrs (2μM dose, AU). Columns are as follows: Chemical name, putative kinase target, Toxic (accessed by substantial (>15%) apoptosis after 12hrs of treatment), IF score (Z-Score of average p53 level after 24hrs of NCS + drug, measured by IF), Live\_Early (Z-Score of average p53 level after 4hrs of NCS + drug), Live\_Late (Z-Score of average p53 level after 22hrs of NCS + drug),

Chemical	Target	Toxic	IF Score	Live_Early	Live_Late
NA NA	0	0.57	0.97	1.1	
(R)- Roscovitine	CDK2	0	0.57	1.4	3
ALW-II-38-3	DDR1	0	0.8	0.9	1.1
ALW-II-49-7	DDR1	0	0.63	1	1.2
AT-7519	CDK9	0	1.2	1.2	2.3
Tivozanib	VGFR2	0	0.62	0.99	1
AZD7762	CHK1	1	nan	nan	nan
AZD8055	mTOR	0	0.18	0.96	0.5
Sorafenib	BRAF	0	0.67	0.92	1.2
CP466722	ATM	0	0.53	1.1	1.1
CP724714	ERBB2	0	0.67	1	0.95
Flavopiridol	CDK1	0	3	1.4	2.4
NA NA	0	0.77	1.1	0.99	
GSK429286A	ROCK1	0	0.77	1.1	0.99
GSK461364	PLK1	1	nan	nan	nan
GW843682	PLK1	0	0.94	1	1.1
HG-5-113-01	LOK	0	0.81	1.1	4.7
HG-5-88-01	EGFR	0	0.71	0.84	0.59
HG-6-64-01	ABL1	1	nan	nan	nan
Neratinib	ERBB2	0	0.77	0.92	0.9
JW-7-24-1	LCK	0	0.78	0.96	0.46
Dasatinib	ABL1	1	nan	nan	nan
Tozaterib	AURKA	0	1	0.93	1.2
GNF2	ABL1	0	0.84	0.85	0.63
Imatinib	ABL1	0	0.68	1.1	1.4
NVP-TAE684	ALK	0	1.2	1.1	0.54
CGP60474	CDK1	1	nan	nan	nan
NA NA	0	0.95	1	1.2	
PD173074	FGFR1	1	nan	nan	nan
Crizotinib	c-Met	1	nan	nan	nan
BMS345541	IKKB	1	nan	nan	nan
GW-5074	c-Raf	0	0.95	1.6	1.7
KIN001-042	GSK3B	0	0.87	1	1.2

KIN001-043	GSK3B	1	nan	nan	nan
Saracatinib	Src	0	0.78	0.94	1
KIN001-055	JAK3	0	0.68	1.1	0.85
AS601245	JNK3	1	nan	nan	nan
Sigma A6730	AKT1	0	0.79	0.83	0.6
SB 239063	MK14	0	0.95	0.96	1.4
NA	NA	0	0.91	0.98	1.2
AC220FLT3		0	0.79	0.79	0.59
WH-4-023	LCK	0	0.8	0.67	0.59
WH-4-025	Src	0	0.95	0.95	0.46
R406	SYK	1	nan	nan	nan
BI-2536	PLK1	1	nan	nan	nan
Motesanib	VGFR1		1	nan	nan
KIN001-127	ITK	0	0.74	1.4	1.8
KIN001-242	RSK2	0	0.58	1.1	1.1
A443654	AKT1	1	nan	nan	nan
SB590885	BRAF	1	nan	nan	nan
GDC-0941	PIK3CA	0	0.46	0.91	0.61
PD184352	MP2K1	0	1.2	0.93	0.85
PLX-4720	BRAF	0	0.89	1.1	1.3
AZ-628	BRAF	0	1.3	0.85	1.3
Lapatinib	EGFR	0	0.69	0.93	0.6
Rapamycin	mTOR	0	0.58	0.95	0.72
NA	NA	0	0.93	0.98	0.94
ZSTK474	PIK3CA	0	0.61	0.83	0.81
AS605240	PIK3CG	0	0.59	0.97	0.88
BX-912	PDK1	0	0.5	1.3	1.1
Selumetinib	MP2K1	0	0.66	1.1	1
MK2206	AKT1	0	0.67	0.64	0.58
CG-930	JNK1	0	0.71	0.96	1.1
AZD-6482	PIK3CB	0	0.66	0.91	0.87
TAK-715	MK14	0	0.98	1	1.4
NU7441	DNA-PK	0	1.1	0.97	1.4
GSK1070916	AURKB	1	nan	nan	nan
OSI-027	mTOR	0	0.54	0.74	0.39
NA	NA	0	0.85	1	1.1
WYE-125132	mTOR	0	0.29	0.89	0.48
KIN001-220	AURKA	1	nan	nan	nan
MLN8054	AURKA	0	0.73	1.1	1.1
Barasertib	AURKB	0	0.53	1.1	1.1
PLX4032	BRAF	0	0.54	0.95	1.1
Enzastaurin	KPCB	1	nan	nan	nan
NPK76-II-72-1	PLK3	1	nan	nan	nan
PD0332991	CDK4	0	0.41	1	0.61
PF562271	FAK	0	0.86	1.3	2.5
PHA-793887	CDK2	0	1.1	1.3	2.4

KU55933	ATM	0	0.42	0.72	0.63		
QL-X-138	BTK	0	0.23	0.49	0.47		
QL-XI-92	DDR1	0	0.65	1.2	0.99		
QL-XII-47	BTK	1	nan	nan	nan		
NA NA	0	0.58	1.1	1			
THZ-2-98-01	IRAK10		0.65	1.2	1.7		
Torin1	mTOR	0	0.4	0.56	0.85		
Torin2	mTOR	1	nan	nan	nan		
KIN001-244	PDK1	0	0.49	1	0.79		
WZ-4-145	CSF1R1		nan	nan	nan		
WZ-7043	CSF1R0		0.6	1.1	0.92		
WZ3105	CLK2	1	nan	nan	nan		
WZ4002	EGFR	0	0.69	1.1	0.7		
XMD11-50	LRRK2		0	0.7	1.4	1.4	
XMD11-85h	BRSK2		0	0.63	1.1	0.69	
XMD13-2	RIPK1	0	0.64	1	1.2		
NA NA	0	0.72	1.1	0.8			
NA NA	0	0.21	1	0.97			
XMD14-99	EPHB3		0	0.25	0.9	0.76	
XMD15-27	CAMK2B		0	0.3	0.99	0.89	
XMD16-144	AURKA		1	nan	nan	nan	
JWE-035	AURKA		1	nan	nan	nan	
XMD8-85	ERK5	0	0.2	1	0.84		
XMD8-92	ERK5	0	0.25	1.1	0.78		
ZG-10	JNK1	1	nan	nan	nan		
ZM-447439	AURKA		0	0.25	0.99	0.89	
Erlotinib	EGFR	0	0.28	0.95	0.89		
Gefitinib	EGFR	0	0.29	1	1		
Nilotinib	ABL1	1	nan	nan	nan		
NA NA	0	0.13	1	0.88			
JNK-9L	JNK1	0	1	1	2.1		
PD0325901	MP2K1		1	nan	nan	nan	
YM 201636	FYV1	0	0.13	0.92	0.6		
FR180204	ERK1	0	0.15	0.96	0.86		
TWS119	GSK3B		0	0.3	1	0.85	
PF477736	CHK1	1	nan	nan	nan		
Kin237	c-Met	0	0.24	0.94	0.68		
Pazopanib hydrochloride			VGFR1	0	0.23	0.99	0.83
LDN-193189	ACVR1		1	nan	nan	nan	
PF431396	FAK	0	0.42	1.3	1.9		
Celastrol	PSB5	1	nan	nan	nan		
Amuvatinib	PGFRA		0	0.24	1.1	0.77	
SU11274	c-Met	1	nan	nan	nan		
Canertinib	EGFR	1	nan	nan	nan		
NA NA	0	0.22	0.99	0.74			
SB525334	TGFR1		0	0.22	1	0.75	

NVP-AEW541		IGF1R	1	nan	nan	nan
SGX523	c-Met	0	0.19	0.98	0.73	
MGCD265	c-Met	1	nan	nan	nan	
PHA-665752	c-Met	0	4	2	1.3	
PI103	PIK3CA	0	0.48	0.92	0.68	
Dovitinib	FLT3	1	nan	nan	nan	
GSK 690693	AKT1	0	0.27	0.98	1.1	
Ibrutinib	BTK	0	0.2	1.1	0.82	
Masitinib	c-Kit	0	0.27	1	0.78	
Tivantinib	c-Met	0	0.26	0.92	0.71	
NA NA	0	0.25	0.98	0.65		
BMS-387032	CDK9	0	1	1.3	1.9	
Afatinib	ERBB2	0	0.4	0.94	0.76	
GSK1904529A		IGF1R	1	nan	nan	nan
OSI 906	IGF1R	0	0.2	1	0.68	
TPCA-1	IKKB	0	0.19	0.96	0.79	
BMS509744	ITK	0	0.26	0.98	1.1	
Ruxolitinib	JAK1	0	0.24	0.93	0.99	
AZD-1480	JAK2	0	0.22	0.95	1.4	
Momelotinib	JAK1	1	nan	nan	nan	
TG 101348	JAK2	1	nan	nan	nan	
Trametinib	MP2K1	0	0.34	1	0.86	
BMS 777607	c-Met	0	0.19	0.97	0.71	
Olaparib	PARP1	0	0.25	1.1	0.96	
Veliparib	PARP1	0	0.28	0.95	0.88	
GSK2126458	PIK3CA	1	nan	nan	nan	
NVP-BKM120		PIK3CA	0	0.21	0.98	0.86
NA NA	0	0.22	0.99	1.1		
XL147	PIK3CA	0	0.21	0.96	0.95	
Y39983	ROCK1	1	nan	nan	nan	
Ponatinib	ABL1	1	nan	nan	nan	
BIBF-1120	VGFR1	1	nan	nan	nan	
MK 1775	WEE1	0	0.23	1.1	1.6	
KIN001-266	M3K8	0	0.19	0.94	0.77	
AT7867	AKT1	1	nan	nan	nan	
KU-60019	ATM	0	0.071	0.78	0.56	
JNJ38877605	c-Met	0	0.21	0.94	0.77	
Foretinib	c-Met	1	nan	nan	nan	
AZD 5438	CDK2	0	0.85	1.2	2.6	
NA NA	0	0.24	1	1.1		
Pelitinib	EGFR	1	nan	nan	nan	
SB 216763	GSK3B	1	nan	nan	nan	nan
NVP-AUY922		HS90A	0	0.16	1.1	1.7
SP600125	JNK1	0	0.24	1.1	2.1	
BIX 02189	MP2K5	0	0.3	0.94	1.3	
AZD8330	MP2K1	0	0.37	1.1	1.2	

PF04217903	c-Met	0	0.21	0.98	0.97	
BAY61-3606	SYK	0	0.55	1	2.1	
SB 203580	MK14	0	0.32	0.94	1.1	
VX-745	MK14	0	0.34	1	1.3	
Doramapimod	MK14	0	0.39	1	1.5	
JNJ 26854165	P53	0	0.33	1.1	1.8	
TGX221	PIK3CB	0	0.24	0.97	1.4	
GSK1059615	PIK3CA	0	0.41	0.98	1.9	
NA	NA	0	0.24	0.99	2	
pseudoXL765	nan	0	0.26	1	1.9	
A 769662	AMPK-alpha1	0	0.26	1	1.9	
Sunitinib malate	VGFR1		1	nan	nan	nan
Y-27632	ROCK1	0	0.26	1.1	1.5	
Brivanib	VGFR1	0	0.26	1.2	1.5	
OSI-930	c-Kit	0	0.39	1.1	1.4	
ABT-737	BCL2	0	0.59	1	1.4	
CHIR-99021	GSK3B	0	0.33	1	1.7	
GDC-0879	BRAF	0	0.22	0.97	2	
Linifanib	FLT3	0	0.49	1	2	
BGJ398	FGFR1	0	0.28	0.92	0.69	